EXHIBIT 2

# Phosphodiesterase 4 Inhibitors for the Treatment of COPD*

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**Phosphodiesterase 4 (PDE4) is a major cyclic adenosine-3′,5′-monophosphate-metabolizing enzyme in immune and inflammatory cells, airway smooth muscle, and pulmonary nerves. Selective inhibitors of this enzyme have been available for a number of years and show a broad spectrum of activity in animal models of COPD and asthma. The class-associated side effects, mainly nausea and emesis, appear to have been at least partially overcome by the so-called "second-generation" PDE4 inhibitors. Currently, three companies are in the later stages of development of candidate second-generation PDE4 inhibitors for the treatment of COPD patients. The preclinical profile of one of these, BAY 19–8004, is summarized below. The initial clinical data on the most advanced compound, cilomilast, were indeed encouraging. However, full knowledge of the therapeutic value of this novel compound class awaits the outcome of longer term clinical trials.**

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**Key words:** BAY 19–8004; cilomilast; COPD; inhibitor; phosphodiesterase; roflumilast; tobacco smoke

**Abbreviations:** Cmax = peak plasma concentration; $ED_{50}$ = median dose for 50% inhibition; LPS = lipopolysaccharide; PDE4 = phosphodiesterase 4

The defining feature of COPD is an accelerated decline in lung function that is, in the overwhelming majority of cases, caused by cigarette smoking and is largely irreversible. Like asthma, COPD is associated with airway inflammation.[1] However, whereas the inflammatory process in asthma can be described simplistically as a CD4+ T-cell-driven eosinophilia, COPD is marked by an increase in the numbers or activity of CD8+ T cells, macrophages, and neutrophils. Long-term trials[2–4] of inhaled corticosteroids in COPD patients have failed to show significant benefit in terms of slowing the progression of this disease. Furthermore, unlike asthma, important elements of this inflammatory response are insensitive to steroids,[5,6] thus highlighting the need for novel anti-inflammatory therapies.

Phosphodiesterase 4 (PDE4) inhibitors have been shown to relax airway smooth muscle, to suppress the activation of inflammatory cells, and to modulate the activity of pulmonary nerves.[7,8] The reported effects of PDE4 inhibitors *in vitro* and in animal models suggest that, in addition to short-term effects on bronchomotor tone, they may find utility in reducing the protease burden associated with neutrophilic inflammation, as well as down-regulating the activity of CD8+ T cells and macrophages. Such effects have the potential to slow the accelerated decline in lung function seen in patients with COPD and, thus, to modify the natural history of this disease.

Despite the wealth of publications documenting the broad anti-inflammatory profile of PDE4 inhibitors *in vitro*, it is worth noting certain limitations that may prove to be of importance in defining their anti-inflammatory impact in the clinical setting. It is clear that some inflammatory cells are less sensitive to PDE4 inhibition than others. Within the same cell type, different functions display different sensitivities to cyclic adenosine-3′,5′-monophosphate elevation. In the neutrophil, degranulation is relatively insensitive compared to superoxide production and leukotriene $B_4$ generation. Thus, some anti-inflammatory effects reported *in vitro* may not be expressed at plasma levels that are attained in humans. A further feature of their profile of activity on neutrophils is that elevation of cyclic adenosine-3′,5′-monophosphate, at least in normal circulating cells, results in delayed apoptosis,[9,10] which could have proinflammatory consequences.

At present, three companies have compounds that are in the late stages of development for COPD treatment (Fig 1). The furthest advanced compound is cilomilast (Ariflo [SB207499]; GlaxoSmithKline; Uxbridge, UK). In a 6-week phase II study[11,12] in patients with moderate COPD, cilomilast caused significant improvements in both lung function and symptom scores at a dose of 15 mg bid. Roflumilast (Byk Gulden; Konstanz, Germany) appears to be the most potent of the three compounds with a dose of 0.5 mg once daily being assessed in phase II. Efficacy has been reported in a phase II study of asthmatic patients,[13] but phase II results for both roflumilast and BAY 19–8004 (Bayer AG; Wuppertal, Germany) in COPD patients have not yet been published.

## Preclinical Profile of BAY 19–8004

### *In Vitro*

BAY 19–8004 is representative of a new structural class of PDE4 inhibitors, the benzofurans. The profile of BAY 19–8004 *in vitro* is summarized in Table 1. In common with cilomilast and roflumilast, it is highly selective for PDE4. The mean concentration required for 50% inhibition of PDE4 that was present in a membrane preparation from human neutrophils was 67 nm. As an inhibitor of other PDE1, -2, -3, and -5 isoform enzymes from a variety of sources, BAY 19–8004 showed < 50% inhibition at a concentration of 10 μM. BAY 19–8004 does not show significant selectivity for a particular subtype of PDE4.

There was a highly significant correlation between potency against this neutrophil PDE4 activity and inhibition of neutrophil superoxide generation for a range of benzofurans. BAY 19–8004 inhibited human inflammatory cell functions that previously were reported to be sensitive to PDE4 inhibitors. It showed some selectivity for neutrophil and eosinophil functions over those of monocytes and T lymphocytes when compared to cilomilast and other reference PDE4 inhibitors. However, this

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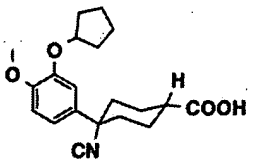
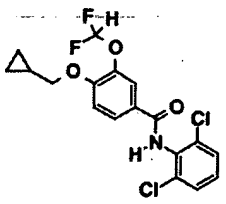
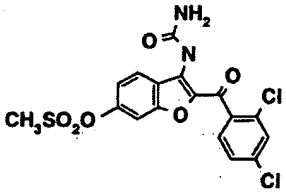
| Compound | Structure | Maximum Dose (Phase II) | Status |
|---|---|---|---|
| Ariflo™ (Glaxo Smith-Kline) |  | 15 mg b.i.d. orally | Phase III |
| Roflumilast (Byk Gulden) |  | 0.5 mg o.d. orally | Phase II |
| BAY 19-8004 (Bayer) |  | 5 mg o.d. orally | Phase II |

FIGURE 1. PDE4 inhibitors in advanced clinical development for COPD.

qualitative difference was not reflected in any differences in the profile of activity between cilomilast and BAY 19–8004 in animal models.

### *In Vivo*

As is expected from this class of compounds, BAY 19–8004 exhibited a broad profile of anti-inflammatory activity in animal models of COPD and asthma (Table 2). It was also an effective bronchodilator. When administered IV to guinea pigs that previously had received aerosol leukotriene $D_4$ to induce a sustained bronchoconstriction, BAY 19–8004 significantly reversed the leukotriene $D_4$-induced response with a median for 50% inhibition dose ($ED_{50}$) of 0.3 to 1 mg/kg. While in standard guinea pig antigen models the $ED_{50}$ of the compound was in the range of 1 to 3 mg/kg, in rat and cynomolgus monkey models the potency was higher, and the associated maximum plasma levels (*ie*, maximum plasma concentration) at the $ED_{50}$ were 60 and 29 ng/mL, respectively.

**Table 1—*Molecular and Cell Function Profile of BAY 19–8004**

| Test | Stimulus | BAY 19–8004 $IC_{50}$, nM |
|---|---|---|
| Human neutrophil PDE4 | NA | 67 |
| [$^3$H]-Rolipram binding (rat brain) | NA | 14 |
| Human neutrophil $O_2^-$ | fMLP | 38 |
| Human neutrophil leukotriene $B_4$ | fMLP | 2 |
| Human eosinophil leukotriene $C_4$ | fMLP | 0.02 |
| Human monocyte TNF-α | LPS | 260 |
| Human lymphocyte interleukin-5 | PHA | 670 |

*fMLP = formyl-methyl-leucyl-phenylalanine; PHA = phytohemagglutinin; $IC_{50}$ = mean concentration required for 50% inhibition; NA = not applicable; TNF = tumor necrosis factor.

The effects of earlier PDE4 inhibitors have been well-documented in the aforementioned models. A feature of COPD that has been less well-studied in relation to PDE4 inhibition is the hypersecretion of mucin. We used a guinea-pig model in which the instillation of bacterial lipopolysaccharide (LPS) results in a sixfold increase in the output of immunoreactive mucin. Pretreatment with BAY 19–8004 (10 mg/kg po) resulted in the complete inhibition of LPS-induced mucin hypersecretion. Inhibition (46%) also was observed with cilomilast at 30 mg/kg po, although this was not statistically significant. Interestingly, the influx of neutrophils in response to LPS was not significantly inhibited in these animals by either PDE4 inhibitor. The inhibition of LPS-induced neutrophil influx in the guinea pig has been reported for cilomilast.[14] The failure to observe this in the present experiments may be due to the higher instillation dose of LPS that is required

Table 2—*Profile of BAY 19–8004 in Animal Models of COPD and Asthma**

| Test | Dose | Plasma Levels, Cmax | Effect |
|---|---|---|---|
| Guinea-pig | | | |
| Antigen-induced bronchoconstriction | 3 mg/kg po | ND | 60% inhibition |
| Antigen-induced eosinophilia | 3 mg/kg po | ND | 63% inhibition |
| Allergic cynomologus monkey | 0.1 mg/kg/d po | 29 ng/mL | 65% inhibition of eosinophil influx; 100% inhibition hyperresponsiveness |
| LPS | | | |
| Rat | 1 mg/kg po | 100 ng/mL | 62% inhibition of neutrophil influx |
| Rat | 10 mg/kg po | ND | 91% inhibition of TNF-α; 89% inhibition of MIP-α |
| Guinea pig | 10 mg/kg po | ND | 100% inhibition of mucin output |
| Tobacco smoke-induced inflammation in guinea pigs | 10 mg/kg po | ND | 100% inhibition of increase in inflammatory cells in BAL fluid |

*ND = not determined; MIP-α = monocyte inhibition protein-α. See Table 1 for other abbreviation.

to elicit mucin hypersecretion. It does, however, suggest that PDE inhibitors could inhibit mucin hypersecretion directly rather than by an indirect mechanism involving neutrophils.

There is currently no information reporting the evaluation of PDE4 inhibition in models of tobacco smoke-induced inflammation and emphysema. In collaboration with Dr. James Hogg (St. Paul Hospital; Vancouver, BC, Canada), we have investigated the effects of BAY 19–8004 in such a model. There is a significant inflammatory response in the lungs of guinea pigs 1 h after exposure to tobacco smoke (*ie*, five cigarettes). The levels of neutrophils, macrophages, and eosinophils all were increased in the BAL fluid. The neutrophil component of this inflammatory response was not inhibited at a dose of 5 mg/kg betamethasone. By contrast, BAY 19–8004 (10 mg/kg po) completely inhibited the influx of all inflammatory cell types. Studies are currently ongoing to determine the effects of BAY 19–8004 on both the inflammatory and emphysematous response that develops after 12 weeks of tobacco smoke exposure.[15] In all efficacy models in which comparisons were performed, BAY 19–8004 was 10- to 30-fold more potent than cilomilast.

## Class-Associated Side Effects

The promise that PDE4 inhibitors will have an improved side-effect profile over nonselective compounds has been borne out in early clinical trials, at least with regard to cardiovascular and most CNS side effects. However, GI side effects, including nausea, vomiting, and dyspepsia, limit the dosages of these compounds that can be administered to humans.[16–18]

The long splice variants of PDE4 can exist as two conformers. One with a high affinity for rolipram predominates in parietal cells and CNS tissue. A second conformer with low affinity for rolipram is present in inflammatory cells. Most, though not all, anti-inflammatory effects are mediated by this form.[19] Reducing activity in the high-affinity form while improving inhibitory potency in the low-affinity form has been suggested as a strategy for improving the therapeutic window of PDE4 inhibitors.[19]

When compared to the archetypal PDE4 inhibitor rolipram, cilomilast shows such an improved relative potency on these two conformers. Nevertheless, at the clinically effective dose (15 mg bid), nausea and headache still were reported, although they were transient in nature. In phase 1 studies, the higher dose of 20 mg bid was reportedly not tolerated.[17]

BAY 19–8004 shows similar relative activities to cilomilast on the two PDE4 conformers (Table 1). Despite the lack of emesis in a ferret model, BAY 19–8004 produced emesis in primates. The threshold dose of BAY 19–8004 for this effect in primates was 10-fold lower than that for cilomilast, suggesting a similar therapeutic window in animal models. The slow absorption of BAY 19–8004 in humans may have an additional benefit with respect to its side-effect profile.

## Summary of Phase 1 Findings With BAY 19–8004

BAY 19–8004 exhibited linear pharmacokinetics with a half-life of 25 h and low plasma clearance in phase 1 studies. There was low intersubject variability. A once-daily administration of 5 mg (the highest dose subsequently used in phase II COPD studies) to elderly patients achieved plasma levels in the range associated with efficacy in animal models (maximum plasma concentration, 68 ng/mL; minimum plasma concentration, 40 ng/mL at steady state). Once-daily administration is therefore the dosing regimen envisaged.

There were no relevant findings with regard to circulation, lung, liver, kidney, or hematology. The most frequently reported adverse event was nausea. The incidence of nausea was dose-related, and, as was reported for cilomilast, it occurred early in the dosing regimen and was transient in nature for most subjects.

To date, there have been no reports regarding the activity of PDE4 inhibitors against features of lung inflammation in either asthmatic or COPD subjects. However, in a recent phase II asthma study, roflumilast provided statistically significant (21%) inhibition of LPS-induced tumor necrosis factor-α production from whole blood *ex*

*vivo*.[20] In phase I studies, we exploited a property of BAY 19–8004 that had not been exhibited by the other PDE4 inhibitors so far examined. The inhibition of superoxide production persists in leukocytes isolated from blood treated with BAY 19–8004. This is despite the fact that this isolation involves at least two hypotonic lysis steps and facilitates the monitoring of systemic anti-inflammatory activity of the compound. A statistically significant (*ie*, up to 46%) dose-related inhibition of leukocyte superoxide production *ex vivo* was observed in phase I volunteers treated with doses up to 15 mg BAY 19–8004 per day.

## What Will Third-Generation PDE4 Inhibitors Look Like?

From the available information on cilomilast, it appears that while the side effects are apparently tolerated at a dose showing efficacy (*ie*, 15 mg bid), they are still a significant problem and almost certainly limit the dose.

Two strategies for further improvement in the therapeutic window of PDE4 inhibitors can be envisaged. The finding that PDE4 exists as four genetically distinct subtypes offers the possibility of identifying subtype-selective inhibitors. There is some evidence that such compounds can target specific inflammatory cell functions.[21,22] However, the selectivity achieved to date has been limited, and there are no published data on the association with side effects. A second approach would be to further reduce potency in the high-affinity conformer. Both cilomilast and BAY 19–8004 represent significant improvements on rolipram in this regard. A compound described in a recent patent suggests that significant further improvement is possible.[23] Whether such a compound will overcome the current dose limitation imposed by the side effects of nausea and emesis remains to be investigated.

## Conclusion

The preclinical data supporting the potential utility of PDE4 inhibitors in COPD are compelling. The observed efficacy of cilomilast in COPD patients also is encouraging. However, it is not clear whether the visible effects on lung function and symptom score are a manifestation of the bronchodilator activity of PDE4 inhibitors or are a consequence of anti-inflammatory effects. Early reports suggest that significant anti-inflammatory effects were not seen. The optimal positioning of PDE4 inhibitors in the treatment of COPD patients is dependent on the demonstration of anti-inflammatory activity. This might predict a beneficial effect on the accelerated rate of lung function decline, which would be a major breakthrough in the treatment of patients with this disease.

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